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1/23

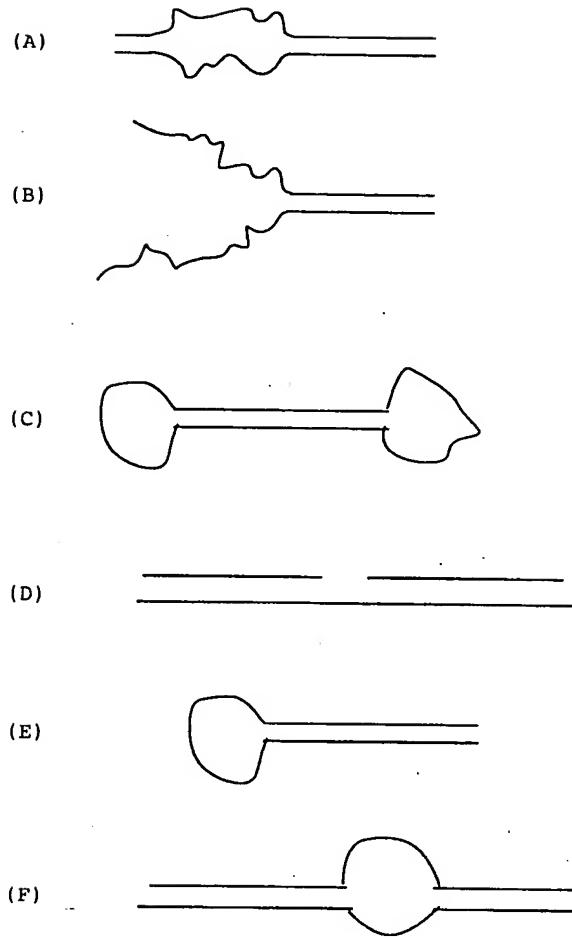


Figure 1 (A-F)

Construct Forms Comprising at Least one Single-Stranded Region

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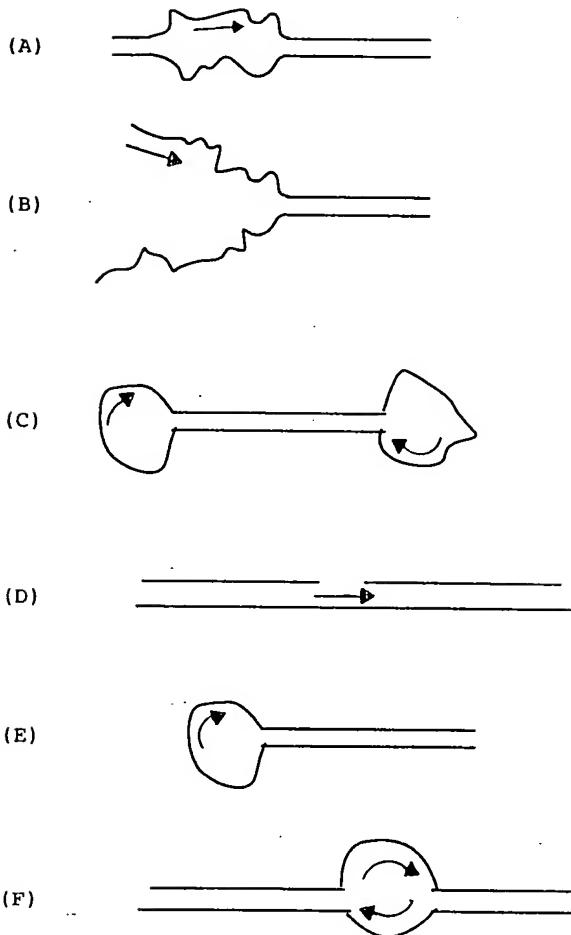


Figure 2 (A-F)

Functional Forms of the Construct

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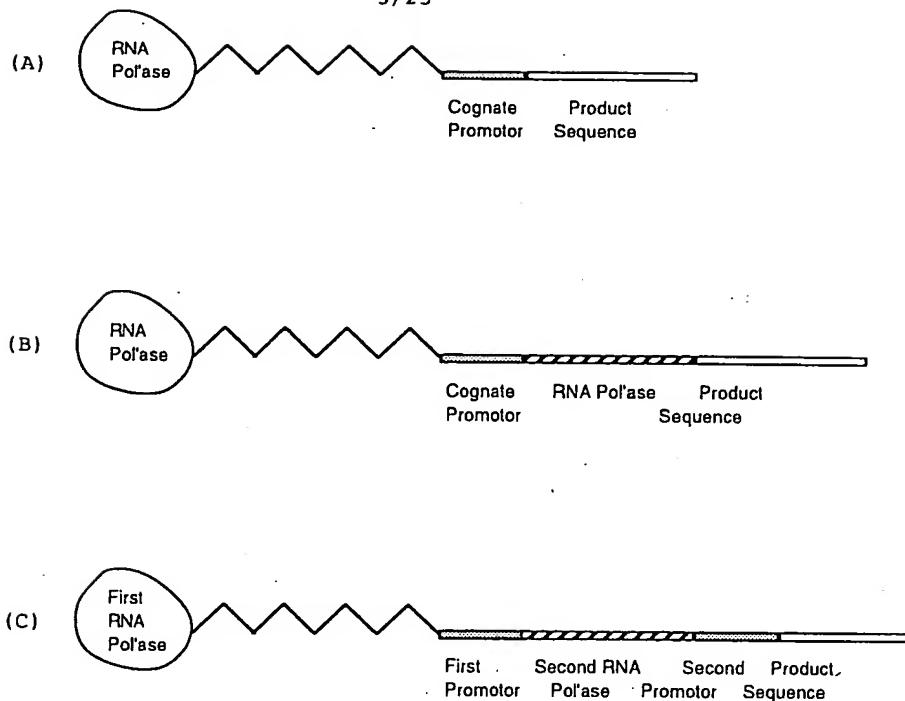


Figure 3 (A-C)

Three Constructs with an RNA Polymerase
Covalently Attached to a Transcribing Cassette

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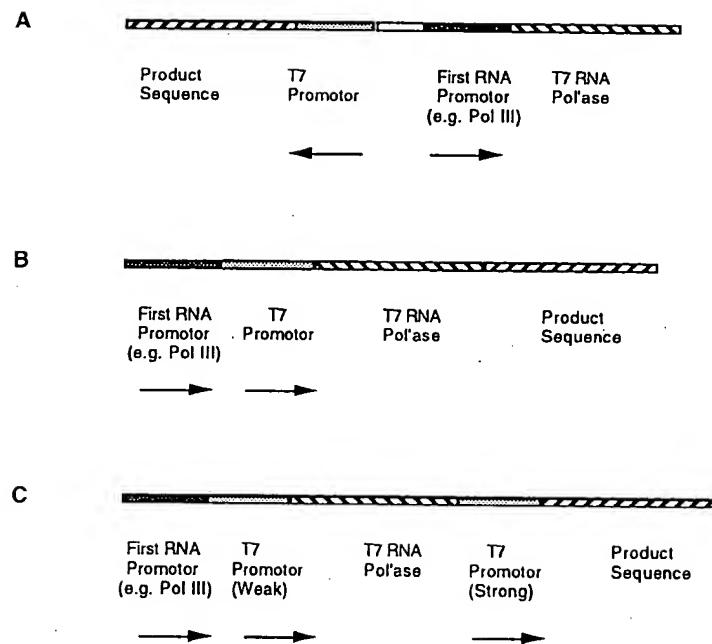


Figure 4 (A-C)

Three Constructs with Promoters
for Endogenous RNA Polymerase

M13mp18. Seq Length: 7250

1. AATGCTACTA CTATTAGTAG AATTGATGCC ACCTTTTCAG CTOGOGOOCC
 51. AAATGAAAAT ATAGCTAAAC AGGTATTGTA CCATTTCGGA AATGTATCTA
 101. ATGGTCAAAC TAAATCTACT CGTTTGCAGA ATTGGGAATC AACTGTTACA
 151. TGGAATGAAA CTTOCAGACA CGTACTTTA GTTGCATATT TAAAACATGT
 201. TGAGCTACAG CACCAGATTG AGCAATTAAG CTCTAACCCA TOCGAAAAAA
 251. TGACCTCTTA TCAAAAGGAG CAATTAAGG TACTCTCTAA TCTTGACCTG
 301. TTGGAGTTTG CTTOCGGTCT GGTTOGCTT GAAGCTGAA TAAAACGCG
 351. ATATTGAAAG TCTTTCGGGC TTCCCTCTTAA TCTTTTGTAT GCAATCGCT
 401. TTGCTTCTGA CTATAATAGT CAGGGTAAG ACCTGATTT TGATTTATGG
 451. TCATTCTCGT TTTCTGAAC TTTAAAGCA TTTGAGGGGG ATTCAATGAA
 501. TATTATGAC GATTOCGCAG TATTGGAOGC TATOCAGTCT AAACATTTA
 551. CTATTACCCC CTCTGGCAAA ACTTCTTTG CAAAGCCTC TCGCTATTT
 601. GGTTTTTATC GTGTTCTGGT AAAAGGGGT TATGATAGTG TTGCTCTTAC
 651. TATGCTCGT AATTCCCTTT GGOGTTATGT ATCTGCATTA GTTGAATGTG
 701. GTATTCTAA ATCTCAACTG ATGAATCTT CTACCTGTAA TAATGTTGTT
 751. COGTTAGTTG GTTTTATTAA CGTAGATTT TCTTCCAAC GTCTGACTG
 801. GTATAATGAG CCAGTTCTTA AAATCGCATA AGGTAATTCA CAATGATTAA
 851. AGTTGAAATT AAACCATCTC AAGOCCAATT TACTACTCGT TCTGGTGTTC
 901. TOGTCAGGGC AAGCTTATT CACTGAATGA GCAGCTTGT TACGTTGATT
 951. TGGGTAAATGA ATATCGGTT CTGTTGAAAG ATTACTCTTG ATGAAGGTCA
 1001 GOCAGCCTAT GOGOCTGGTC TGTACACCGT TCATCTGTOC TCTTCAAAG
 1051 TTGGTCAGTT CGGTTCCCTT ATGATTGACC GTCTGOGOCT CGTTCOGGCT
 1101 AAGTAACATG GAGCAGGTG CGGATTTOGA CACAATTTAT CAGGOGATGA
 1151 TACAATCTC CGTTGTACCTT TGTTTGGCG TTGGTATAAT CGCTGGGGGT
 1201 CAAAGATGAG TGTTTGTG TATTCTTCTG CCTCTTTGCG TTAGGTTGG

Figure 5

M13mp18 Nucleic Acid Sequence

1251	TGCGCTTGT	GTGGCATTAC	GTATTTTAC	CGTTTAATGG	AAACTTTC
1301	ATGAAAAAGT	CTTTAGTCT	CAAAGCCT	GTAGCGGTG	CTACCCCTG
1351	TOCGATECTG	TCTTTCGCTG	CTGAGGGTGA	CGATOOOGCA	AAAGGGGCT
1401	TTAACCTCCT	GCAAGCCTCA	GCGACCGAAT	ATATCGGTTA	TGCGTGGGCG
1451	ATGGTTGTTG	TCATTGTCGG	CGCAACTATC	GGTATCAAGC	TGTTTAAGAA
1501	ATTCAACCTCG	AAAGCAAGCT	GATAAAACGA	TACAATTAAA	GGCTCCTTT
1551	GGAGCCTTT	TTTTGGAGA	TTTCAACGT	GAAAAAATTA	TTATTOGCAA
1601	TTCCTTGT	TGTTCTTTC	TATTCTCACT	CGCTGAAAC	TGTTGAAAGT
1651	TGTTTAGCAA	AACCCCATAC	AGAAAATCA	TTTACTAACG	TCTGGAAAGA
1701	CGACAAAAC	TTAGATCGTT	ACGCTAACTA	TGAGGGTTGT	CTGTGGAATG
1751	CTACAGGCGT	TGTTAGTTGT	ACTGGTGAOG	AAACTCAGTG	TTACGGTACA
1801	TGGGTTCTA	TGGGCTTGC	TATCCCTGAA	AATGAGGGTG	GTGGCTCTGA
1851	GGGTGGGGT	TCTGAGGGTG	GGGGTCTGA	GGGTGGGGT	ACTAAACCTC
1901	CTGAGTAOGG	TGATACACCT	ATTCGGGCT	ATACTTATAT	CAACCCCTCTC
1951	GAOGGCAC	ATCGCGCTGG	TACTGAGCAA	AACCGCTA	ATCCTAATCC
2001	TTCCTCTGAG	GAGTCTCAGC	CTCTTAATAC	TTTCATGTT	CAGAATAATA
2051	GGTTCGAAA	TAGGCAGGGG	GCATTAAC	TTTACCGGC	CACTGTTACT
2101	CAAGGCAC	ACCCCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGCCATG	TATGACGCTT	ACTGGAAOGG	TAAATTCAAGA	GAATGCGCTT
2201	CAAGGCAC	ACCCCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2251	AAAAGCCATG	TGCGCTCAACC	TCCTGTCAAT	CGTGGGGGCG	GCTCTGGTGG
2301	TOGTCCTGAG	CTTTAATCAA	GATCCATTG	TTTGTGAATA	TCAAGGCCAA
2351	TGGTCTCTGGT	GGGGGCTCTG	AGGGTCTGG	CTCTGAGGGT	GGCGGTTCTG
2401	AGGGTGGGGG	CTCTGAGGGG	GGGGGTTGG	GTGGTGGCTC	TGGTTCOGGT
2451	GATTITGATT	ATGAAAAGAT	GGCAAACGCT	AATAAGGGGG	CTATGACCGA
	AAATGCGAT	GAAGACGCGC	TACAGTCTGA	CGCTAAAGGC	AAACTTGATT

Figure 5

M13mp18 Nucleic Acid Sequence

2501	CTGTGCGTAC	TGATTACGGT	GCTGCTATCG	ATGGTTTCAT	TGGTGACGTT
2551	TCGGGCGCTTG	CTAATGGTAA	TGGTGCTACT	GGTGATTTTG	CTGGCTCTAA
2601	TTCCCAAATG	GCTCAAGTGC	GTGACGGTGA	TAATTCAACCT	TTAATGAATA
2651	ATTTCCGTCA	ATATTTACCT	TOCCCTCCTC	AATOGGTTGA	ATGTGCGOCT
2701	TTTGTCTTTA	GGCGCTGGTAA	ACCATATGAA	TTTCTATTG	ATTGTGACAA
2751	AATAAACTTA	TTCGGTGGTG	TCTTTCGCGTT	TCTTTTATAT	GTGCGACCT
2801	TTATGTATGT	ATTTTCTACG	TTTGCTAAC	TACTGCGTAA	TAAGGAGTCT
2851	TTATCATGOC	AGTTCTTTG	GGTATTGCGT	TATTATTGCG	TTTCTCGGGT
2901	TTCCTCTGG	TAACCTTGTT	CGGCTATCTG	CTTACTTTTC	TTAAAAAGGG
2951	CTTGGTAAG	ATAGCTATTG	CTATTCATT	GTTCCTTGCT	CTTATTATTG
3001	GGCTTAACTC	AATTCTTG	GGTTATCTCT	CTGATATTAG	CGCTCAATTA
3051	CCCTCTGACT	TTGTTCAAGGG	TGTTCAAGTTA	ATTCCTCGT	CTAATGCGCT
3101	TCCCTGTTT	TATGTTATTG	TCTCTGTAAA	GGCTGCTATT	TTCATTTTG
3151	ACGTTAAACA	AAAAATCGTT	TCTTATTG	ATGGGATAA	ATAATATGGC
3201	TGTTTATTG	GTAACGGCA	AATTAGGCTC	TGGAAAGAOG	CTGGTTAGOG
3251	TTGGTAAGAT	TCAGGATAAA	ATTGAGCTG	GGTGCAAAT	AGCAACTAAT
3301	CTTGATTAA	GGCTTCAAAA	OCTCCCGCAA	GTOGGGAGGT	TCGCTAAAC
3351	GGCTGGCGTT	CTTAGAATAC	CGGATAAGCC	TTCTATATCT	GATTTGCTTG
3401	CTATGGGCG	CGGTAATGAT	TOCTACGAATG	AAAATAAAA	CGGCTTGCTT
3451	GTTCCTOGATG	AGTGCGGTAC	TTGGTTTAAT	ACCGGTTCTT	GGAATGATAA
3501	GGAAAGACAG	CGGATTATTG	ATTGGTTCT	ACTGCTG	AAATTAGGAT
3551	GGGATATTAT	TTTCTTGTT	CAGGACTTAT	CTATTGTTGA	TAACAGGGG
3601	CGTTCTGCAT	TAGCTGAACA	TGTTGTTTAT	TGTOGTOGTC	TGGACAGAAT
3651	TACTTACCT	TTTGTGGTAA	CTTATATTG	TCTTATTACT	GGCTGAAAAA
3701	TGCGCTCTGCG	TAAATTACAT	GTGGGCGTTG	TTAAATATGG	CGATTCTCAA
3751	TTAAGGCGTA	CTGTTGACCG	TTGGCTTAT	ACTGGTAAGA	ATTTGTATAA
3801	CGCATATGAT	ACTAAACAGG	CTTTTCTAG	TAATTATGAT	TOCGGTGTTT

Figure 5

M13mp18 Nucleic Acid Sequence

3851	ATTCTTATTT	AACGGCTTAT	TTATCACACG	GTCGGTATT	CAAACCATT
3901	AATTTAGGTC	AGAAGATGAA	ATTAACAAAA	ATAATATTGA	AAAAGTTTC
3951	TGCGGTTCTT	TGTCTTGC	TTGGATTTC	ATCAGCATTT	ACATATAGTT
4001	ATATAACCCA	ACCTAACGCG	GAGGTAAAAA	AGTAGTC	TCAGACCTAT
4051	GATTTTGATA	AATTCACTAT	TGACTCTTCT	CAGCGTC	ATCTAAGCTA
4101	TGCTATGTT	TTCAAGGATT	CTAAGGGAAA	ATTAATTAAT	AGCGAOGATT
4151	TACAGAAGCA	AGGTTATTCA	CTCACATATA	TTGATTATG	TACTGTTCC
4201	ATAAAAAAAG	GTAATTCAAA	TGAAATTGTT	AAATGTAATT	AATTTGTTT
4251	TCTTGATGTT	TGTTTCATCA	TCTTCTT	CTCAGGTAAT	TGAAATGAAT
4301	AATTGCGCTC	TGCGCGATT	TGTAACCTGG	TATTCAAAGC	AATCAGGCGA
4351	AATCCGTTATT	GTTTCTOOG	ATGTAAAAGG	TACTGTTACT	GTATATTCAT
4401	CTGACGTTAA	ACCTGAAAAT	CTACGCAATT	TCTTATTT	TGTTTACGT
4451	GCTAATAATT	TTGATAATGGT	TGGTTCAATT	CCTCCATAA	TTCAGAAGTA
4501	TAATCCAAAC	AATCAGGATT	ATATTGATGA	ATTGOCATCA	TCTGATAATC
4551	AGGAATATGA	TGATAATTCC	GCTCTCTG	GTGGTTCTT	TGTTCOGCAA
4601	AATGATAATG	TTACTCAAAC	TTTAAAATT	AATAACGTT	GGGCAAAGGA
4651	TTAATACGA	GTTGTOGAAT	TGTTGTAAA	GTCTAAACT	TCTAAATCCT
4701	CAAATGTATT	ATCTATTGAC	GGCTCTAAAC	TATTAGTTGT	TAGTGCTOCT
4751	AAAGATATT	TAGATAACCT	TCCTCAATT	CTTCTACTG	TTGATTGOC
4801	AACTGACCG	ATATTGATTG	ACGGTTGAT	ATTTGAGGTT	CAGCAAGGTG
4851	ATGCTTCTAGA	TTTTTCATT	GCTGCTGGCT	CTCAGGTTG	CACTGTTGCA
4901	GGGGGTGTTA	ATACTGACCG	CCTCAOCTCT	GTTTTATCTT	CTCTGGTGG
4951	TTCGTTGGT	ATTTTAATG	GCGATGTTT	AGGCTATCA	GTTCGOGCAT
5001	TAAAGACTAA	TAGCCATTCA	AAAATATTGT	CTGTCGCAOG	TATTCTTACG
5051	CTTCAGGTC	AGAAGGGTC	TATCTCTGTT	GGCGAGATG	TCCCTTTAT
5101	TAAAGACTAA	TAGCCATTCA	AAAATATTGT	CTGTCGCAOG	TATTCTTACG
5151	CGATTGAGCG	TCAAAATGTA	GGTATTCCA	TGAGCGTTT	TOCTGTTGCA

Figure 5

M13mp18 Nucleic Acid Sequence

5201	ATGGCTGGCG	GTAATATTGT	TCTGGATATT	ACCAGCAAGG	CGGATAGTTT
5251	GAGTTCTCT	ACTCAGGCAA	GTGATGTTAT	TACTAATCAA	AGAAGTATTG
5301	CTACAAACGGT	TAATTTGCGT	GATGGACAGA	CTCTTTTACT	CGGTGGCGTC
5351	ACTGATTATA	AAAACACTTC	TCAAGATTCT	GGCGTACCGT	TOCTGTCTAA
5401	AATCCCTTTA	ATOGGCGCTC	TGTTTAGCTC	CGCGCTCTGAT	TOCAAACGAGG
5451	AAAGCACGTT	ATACGTGCTC	GTCAAAGCAA	CCATAGTAAG	CGCGCTGTAG
5501	CGGCGCATTA	AGCGCGGGGG	GTGTGGTGGT	TAOGCGCAGC	GTGACCGGCTA
5551	CACTTGCGAG	CGCGCTAGCG	CGCGCTCTT	TOGCCTTCTT	CGCTTCTTCTT
5601	CTCGCGACG	TOCGCGCTT	TOCGCGCTAA	GCTCTAAATC	GGGGCGCTOC
5651	TTTGGGGTTC	CGATTTAGTG	CTTAAAGCA	OCTOGACOOC	AAAAAAACTTG
5701	ATTTGGGTGA	TGGTTCACGT	AGTGGCGCAT	CGCGCTGATA	GACGGTTTTT
5751	CGCGCTTGTG	CGTTGGAGTC	CAOGTTCTT	AATAGTGGAC	TCTTGTGCGA
5801	AACTGGAAACA	ACACTCAACC	CTATCTGGG	CTATTCTTT	GATTITATAAG
5851	GGATTTGCC	GATTIOGGAA	CGACCATCAA	ACAGGATTT	CGCGCTCTGG
5901	GGCGAACACG	CGTGGACCGC	TTCCTGCAAC	TCTCTCAGGG	CGACGGGGTG
5951	AAGGGCAATC	AGCTGTTGCC	CGTCTCGTG	GTGAAAAGAA	AAACCAACCT
6001	GGCGCGCAAT	AOGCAAACCG	OCTCTCCCG	CGCGTGGCC	GATTCACTAA
6051	TGCACTGCG	ACGACAGGTT	TOCGACTGG	AAACCGGGCA	GTGAGCGCAA
6101	CGCAATTAAAT	GTGAGTTAGC	TCACTCATTA	GGCACCCAG	GCTTTACACT
6151	TTATGCTTCC	GGCTCGTATG	TTGTGTGGAA	TTGTGAGGG	ATAACAAATT
6201	CACACAGGAA	ACAGCTATGA	CGATGATTAC	GAATTGAGC	TOGGTACCOG
6251	CGCGATCTCT	AGAGTCGACC	TGCAAGGCGATG	CAAGCTTGGC	ACTGGCGTC
6301	GTTTACAAC	GTGCGTGAATG	GGAAAAACCT	CGCGTAAACG	AACTTAAATG
6351	OCTTGAGCA	CAATCCCCTT	TOCGACGCTG	CGCGTAAATGC	GAAGAGGCGC
6401	GCACCGATCG	CGCTTCCCAA	CAGTTGCGCA	CGCGTAAATGG	CGAATGGCGC
6451	TTTGCCTGGT	TTTGGCGCAOC	AGAAGGGTG	CGCGAAAGCT	CGCTGGAGTG
6501	CGATCTTCT	GAGGCGATA	CGCGTACGCGT	CGCGCTCAAAC	TGGCAGATGC

Figure 5

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6551	ACGGTTAOGA	TGCGGCCATC	TACACCAAOG	TAACCTATCC	CATTACGGTC
6601	AATCGCGGT	TTGTTCCCAC	GGAGAAATCG	ACGGGTTGTT	ACTCGCTCAC
6651	ATTTAATGTT	GATGAAAGCT	GGCTACAGGA	AGGCAGACG	CGAATTATTT
6701	TTGATGGCGT	TCCTATTGGT	TAAAAAATGA	GCTGATTTAA	CAAAAATTTA
6751	ACGCGAATT	TAACAAAATA	TTAACGTTA	CAATTAAAT	ATTGCTTAT
6801	ACAATCTTCC	TGTTTTGGG	GCTTTCTGA	TTATCAACCG	GGGTACATAT
6851	GATTGACATG	CTAGTTTAC	GATTACCGT	CATCGATTCT	CTTGTTTGCT
6901	CCAGACTCTC	AGGCAATGAC	CTGATAGCT	TTGTAGATCT	CTCAAAAATA
6951	GCTACOCTCT	COGGCATGAA	TTTATCAGCT	AGAAOGGTG	AATATCATAT
7001	TGATGGTGAT	TTGACTGTCT	COGGCCCTTC	TCACCCCTTT	GAATCTTAC
7051	CTACACATTA	CTCAGGCATT	GCATTTAAA	TATATGAGGG	TTCTAAAAAT
7101	TTTTATCCTT	GGTTGAAAT	AAAGGCTTCT	COOGCAAAAG	TATTACAGGG
7151	TCATAATGTT	TTTGGTACAA	CCGATTAGC	TTTATGCTCT	GAGGCTTAT

Figure 5

M13mp18 Nucleic Acid Sequence

COMPLEMENTARY TO M₁₃

POSITION 645	5' AGCAACACTATCATA 3'	POSITION 631	M ₁₃ /1
615	ACGAOGATAAAAACC	601	M ₁₃ /2
585	TTTGCAAAAGAAGT	571	M ₁₃ /3
555	AATAGTAAAATGTTT	541	M ₁₃ /4
525	CAATACTGCGGAATG	511	M ₁₃ /5
495	TGAATCCOOCCTCAAA	481	M ₁₃ /6
465	AGAAAACGAGAACATGA	451	M ₁₃ /7
435	CAGGTCTTTACOCTG	421	M ₁₃ /8
405	AGGAAGOGGGATTGC	391	M ₁₃ /9
375	AGGAAGOOOGAAAGA	361	M ₁₃ /10

COMPLEMENTARY TO SS PHAGE DNA

POSITION 351	5' ATATTTGAAGTCTTT 3'	POSITION 366	M ₁₃ /11
371	TCTTTTGATGCAAT	386	M ₁₃ /12
391	CTATAATACTCAGGG	406	M ₁₃ /13
411	TGATTTATGGTCATT	426	M ₁₃ /14
431	GTTTAAAGCATTG	446	M ₁₃ /15
451	TATTTATGACGATT	466	M ₁₃ /16
471	TATCCAGTCTAAACA	486	M ₁₃ /17
491	CTCTGGCAAAACTTC	506	M ₁₃ /18
511	TCGCTATTTGGTTT	526	M ₁₃ /19
531	AAACGAGGGTTATGA	546	M ₁₃ /20

Figure 6

Primers for Nucleic Acid Production
Derived from M13mp18 Sequence

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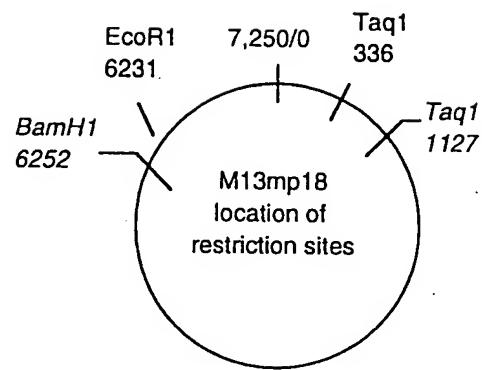
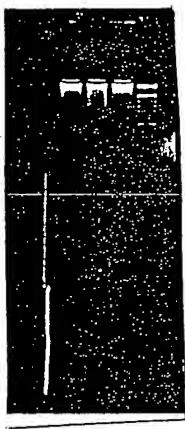


Figure 7

Appropriate M13mp18 Restriction Sites

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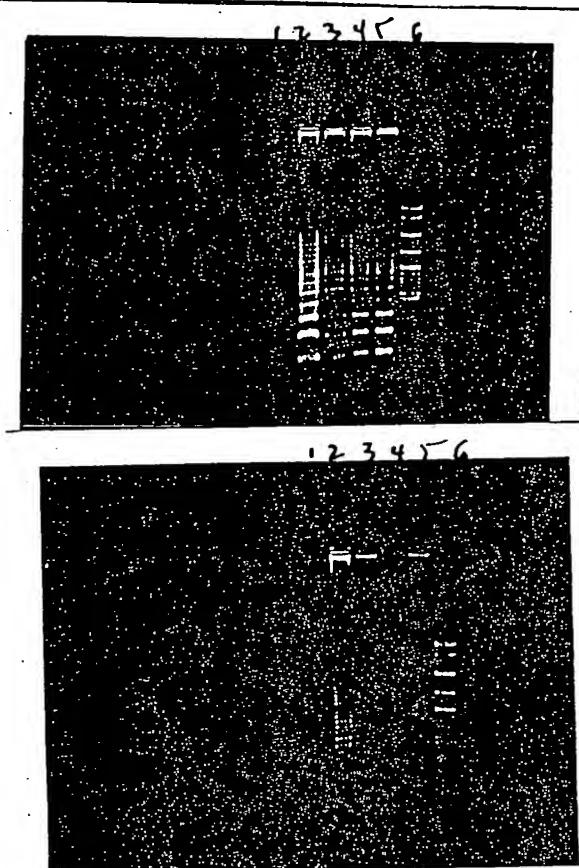
Lane 1: from calf thymus + Taq digested mp18 amplification reaction
Lane 2: from Taq digested mp18 amplification reaction
Lane 3: from calf thymus amplification reaction
Lane 4: øX174 Hinf1 size marker

Figure 8



- Lane 1: no template
- Lane 2: mp18 template, phosphate buffer
- Lane 3: Mspl/pBR322 size marker
- Lane 4: mp18 template, MOPS buffer

Figure 9

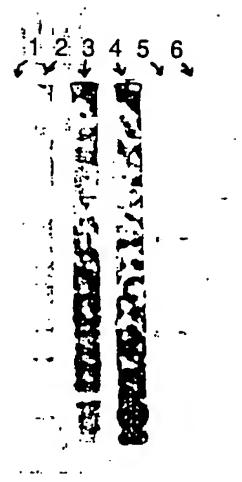


Top= (+) Template
Bottom= (-) Template

- Lane 1: phosphate buffer
- Lane 2: MES
- Lane 3: MOPS
- Lane 4: DMAB
- Lane 5: DMG
- Lane 6: pBR322/MspI size marker

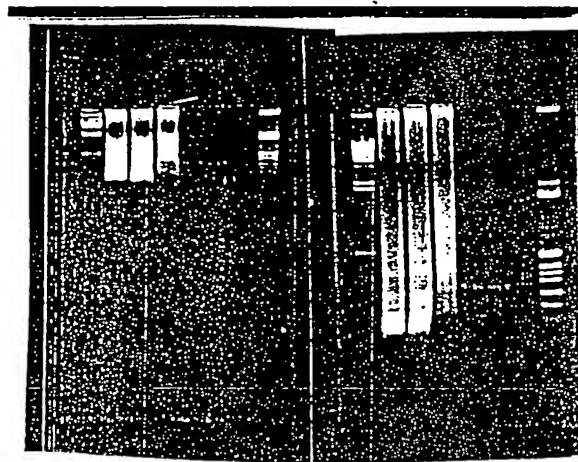
Figure 10

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- Lane 1: DMAB buffer, no template
- Lane 2: DMAB buffer, mp18 template
- Lane 3: DMG buffer, no template
- Lane 4: DMG buffer, mp18 template
- Lane 5: No reaction
- Lane 6: 200 ng Taq I digested mp18
size marker/positive control

Figure 11



First Time Interval Second Time Interval

Agarose Gel Analysis

- Lane 1: lambda Hind III marker
- Lane 2: Amp/Untreated
- Lane 3: Amp/Kinased
- Lane 4: Amp/Kinased/Ligated
- Lane 5: PCR/Untreated
- Lane 6: PCR/Kinased
- Lane 7: PCR/Kinased/Ligated
- Lane 8: øX174/Hinf1 marker

Figure 12

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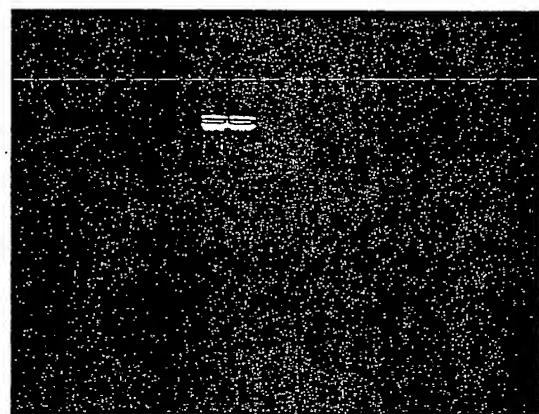
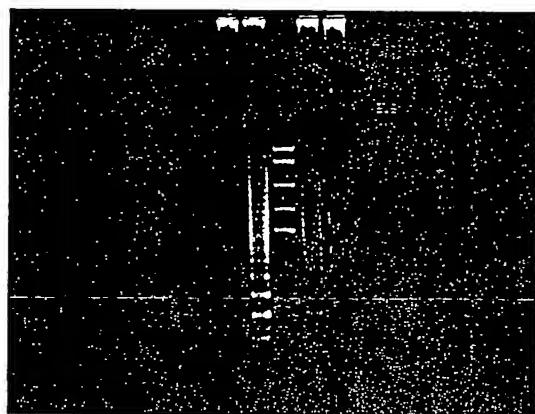


Figure 13

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1 2 3 4 5 6



Lane 1: Primers alone
Lane 2: Primers + taq digested M13 DNA
Lane 3: Molecular weight markers
Lane 4: Primers + RNA
Lane 5: Primers alone
Lane 6: M13 digested DNA
Buffer was dimethyl amino glycine, pH 8.6

Figure 14



Lane 1: Primers alone
Lane 2: Primers + taq digested M13 DNA
Lane 3: Molecular weight markers
Lane 4: Primers + RNA
Lane 5: Primers alone
Lane 6: M13 digested DNA
Buffer was dimethyl amino glycine, pH 8.6

Figure 15

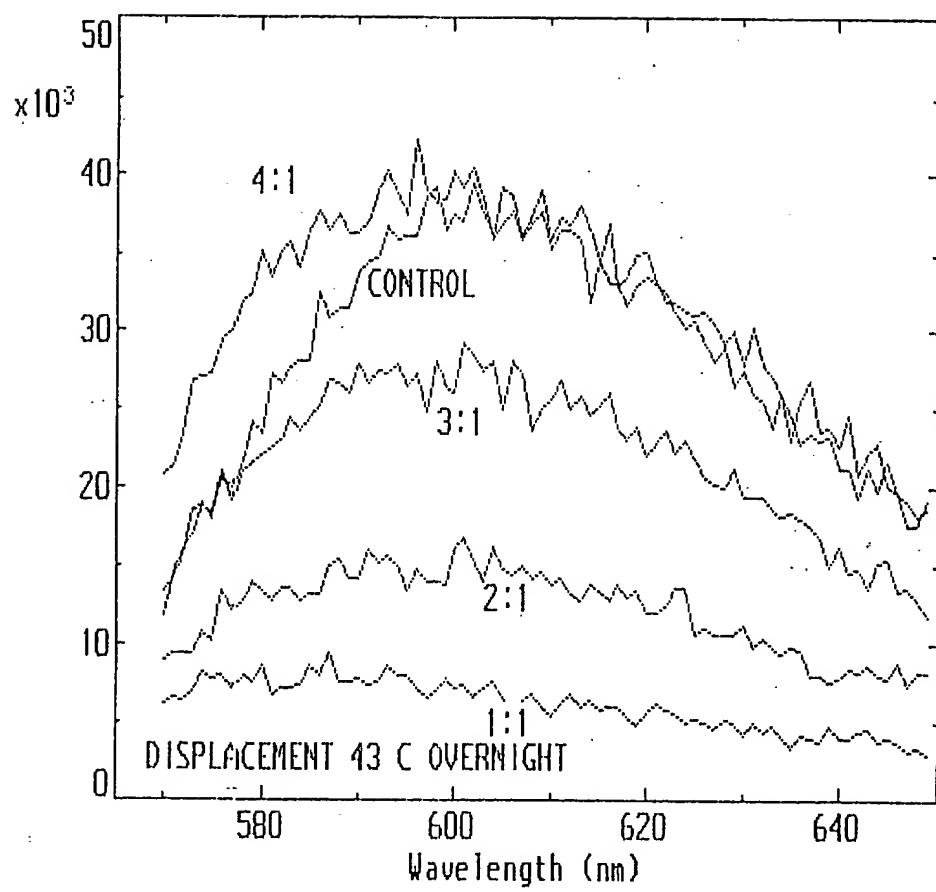


Figure 16

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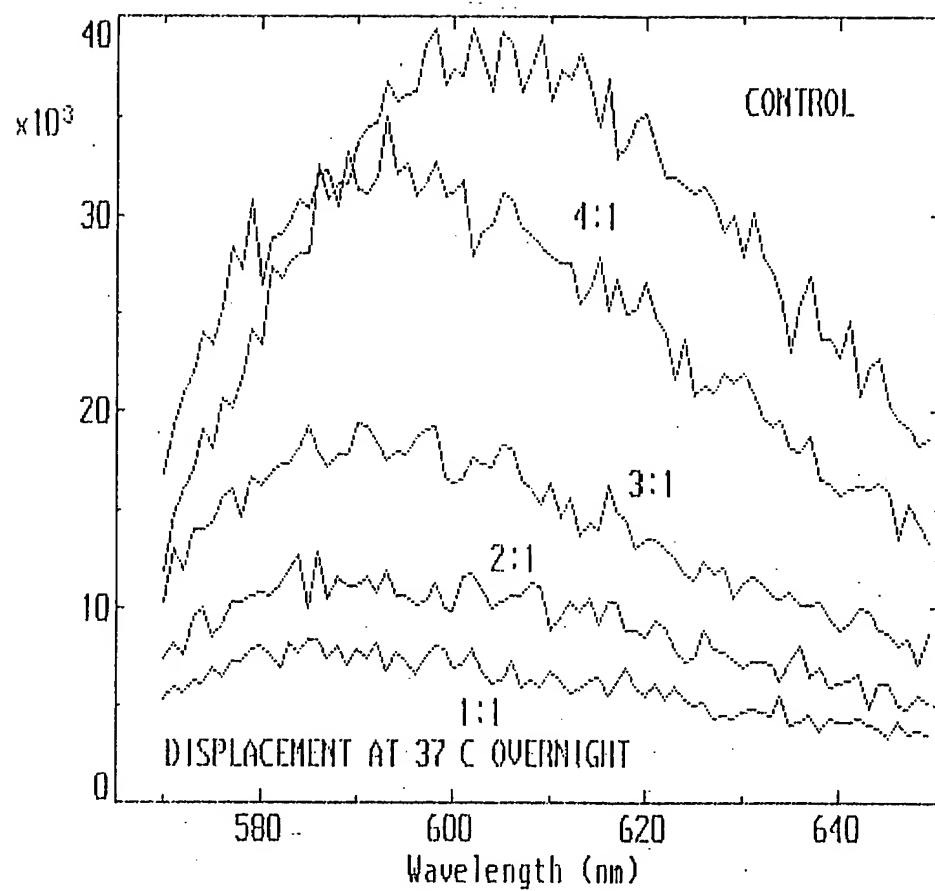


Figure 17

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pIBI 31-BH5-2

fmet AUG of Lac z [T7 Promotor region...]
LAC PROMOTOR..ATG ACC ATG ATT ACG CCA GAT ATC AAA TTA ATA CGA CTC ACT ATA
oligo 50-mer 3'- tac t'aa t'gc ggt* ct'a t'ag t'Vt aat* tat* gct* gag t'ga t'at* c-5'
10 base insert
T7 RNA Start {““ T3 Promotor Region)
IGGG CTC ICCT TTA GTG ACG GTT AAT
““““} “- T3 Start Signal

pIBI 31 BSII/HCV

fmet AUG of Lac z [T3 Promotor region --] T3 RNA Start
LAC PROMOTOR ..ATG ACC ATG ATT ACG CCA AGC TCG AAA TTA ACC CTC ACT AAA /GGG
oligo 50-mer 3'- tac t'aa t'ac t'aa t'gc ggt* t'V--10 base insert!-----
(“- T7 Promotor Region)
MULTIPLE CLONING SITE + 390 BASE INSERT CTA /TAG TGA GTC CGT ATT AAT....
“- T7 Start Signal
5'-ct'a t'ag t'ga gt*c gt'a tt'a at'.....

Figure 18